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Fortified and bioengineered yoghurt: prospects for bacteriotherapy and nutrient deficiency management

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Abstract

One of the main vital processes for the enhancement of food nutrition quantity and quality is food fortification and bioengineering. Global utilization and functional properties of probiotics from yoghurts, make it a good candidate for fortification and bioengineering. Yoghurt is the most popular fermented milk product that is biochemically altered by activities of special microorganisms to achieve desired functional and sensory properties. Due to global food crisis, and health challenges from metabolic disorders and drug resistance. The prospect of fortified and bioengineered yoghurt as an effective bacterio-therapy and nutrient deficiencies management approach is new trend among stakeholders. The aim of this review is to underscore the prospect of fortified and bioengineered yoghurt for the bacterio-therapy and nutrition deficiency management. In this review paper fortification and bioengineering was defined and the major aims of prospect for bacterio-therapy and nutrition deficiency management were presented. Yoghurt fortified with different vitamins and minerals ingredients that exhibit functional properties were discussed. The main problems for human health in today's world is infectious diseases, so yearly gastrointestinal infections resulted to sickness and death significantly worldwide. Therefore, drinking of fortified and bioengineered fermented dairy products increase host metabolism by keeping the balance of intestinal micro flora and thus on the host have a positive effect. The new dairy products designed to be fortified and bioengineered can resolved the problems of nutritional deficiencies. However, fortification of yoghurt with elemental and micronutrients are still developing challenges due to oxidation, solubility and sensory acceptance. Likewise, all genetically modified organisms products (GMO), acceptance of bioengineered yoghurts is still a surmountable challenge with adequate education.

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1.0 Introduction

Yoghurt is the products of milk that broadly undergo changes biochemically as a result of the act of microorganism to provide long shelf life for perishable foods and to increase the flavor and aroma of final food products consumed worldwide (Buckenhüskes,1993). The yoghurt production as fermented product started in the Middle East that spread worldwide. Fermentation is chemical activity in which enzymes break the organic constituents into minor composites. Because of fermentation more flavored foods, stable and digestible with improve nutritional value is produced. The microorganisms responsible for fermentation include yeast, bacteria or molds, while during their growth fermented foods are produced (Yousef, 2003). Fermented milk products produced by *Lactobacillus bulgaricus* and *Streptococcus thermophiles* is called yoghurt. The significance of food quality and safety is considered; therefore more thought is being given to the consumers' health (Grunert, 2005). Because of deficiencies of nutrients in human societies particularly in specific times of life, importing, approval and fortified food consumption is increasing

(Preedy *et al*., 2013). The addition of more or one vital elements to food increased their concentration in that specific food to higher levels than the normal concentration is called fortification which is meant to stopping and adjusting deficiencies in one or more food nutrients in society (Bonner *et al*., 1999). The nutrition researchers said food products fortification using natural resources like cereal, fruit will be one of the major ways to increase the intake of overall food nutrient with least side effect of contamination with toxic compounds (Nestle, 2013). Although, the records on the production of food products fortified with natural resources for purpose of consumers' health and absence of toxicity resulting from use of this material look critical. When yoghurt is compared to milk, it is more nutritious with essential source of calcium, protein, riboflavin, phosphorus, vitamin B12, thiamin, zinc, magnesium, folate and niacin. Bioengineered yoghurt is the products that is produced with genetically modified microorganisms. Most genetically engineered bacteria in yoghurt products are for therapeutic purpose and needs to be consumed in adequate quantities to deliver systematically the products of the genes engineered. Therapeutic function of anaerobic and facultative anaerobic bacteria in yoghurt are specially targeted to colonize necrotic and hypoxic regions like those found in tumors (Baban *et al*.,2010). Furthermore, ingestion of fermented milk products like yoghurt causes stomach pH minor reduction that reduces pathogen transfer risk with effects of the low gastric juice secretion problem (O'connell and Fox**,** 2001**)**. However, since the products of milk fermented are food consumed greatly in the world, fermented products have been used to supply components of nutrition into human diet, also products fortification like yoghurt products is a good method to increase intake of nutrient in daily food products (Grunert, 2005).

2.0 Yoghurt fortification with iron

The fortification of yoghurt with iron is vital as a basis of calcium and protein (Hashemi *et al*., 2015) but products of milk are deficient in iron and other minerals like zinc, nickel, copper and silver. Products of Dairy fortification with iron can improve nutritional deficiencies, while fortified yoghurt with iron has a high bioavailability of iron (Woestyne *et al*., 1991). The effects of iron to be added to yoghurt must be assayed before performing any fortification process. Certain parameters of fortified yoghurt that must be ascertain include taste, oxidation of fat, microbial physiology, sensory, shelf life and overall acceptability of the products is very important (Zhang, 1990). The fortified products of dairy properties are influenced by source of mineral type and component quantity added to the product. Fortified yoghurts produced two key off flavors like metallic flavor and oxidized flavor that is due to the catalytic iron and iron salts role separately (Jackson and Lee, 1991). The fat oxidation happens in milk and yoghurt fortified with ammonium, ferric and ferrous sulphate (Hashemi *et al*., 2015) reduced this element absorption.. (Hashemi *et al*., 2015)noted that oxidation of fat in chocolate milk and related products was not stimulated by fortification with a whey protein complex ferric phosphate compared to other sources and types of ferric salts. The chocolate milk was fortified by whey protein complex ferric phosphate had acceptable flavor properties while other products with ferrous gluconate or ferric chloride was not accepted. Though the ferric ammonium citrate rises milk oxidation but does not occurred in solid dairy product like in cottage cheese (Hashemi *et al*., 2015). Some studies have shown that lipid oxidation was reduced by encapsulated iron than un-capsulated iron in fortified yoghurt, through the experiment lasted for three weeks only. Also yoghurt fortified with C vitamin had acceptable sensory parameters. This demonstrates that iron microcapsules and vitamin C fortification are efficient means to fortify product of dairy without significant changes in their sensory properties (Kim *et al*., 2003). Fortification of yoghurt with iron can be essential and successful policy to control deficiency of iron that cause anemia but adding iron to yoghurt is still developing challenge. The ferrous sulfate related to iron compounds which are insoluble in water are absorbed less. But their benefit as fortification for yoghurts have risen in the past particularly because of the young target. The ferric pyrophosphate is one of the compounds for numerous reasons have been assayed widely in numerous products that are difficult to fortify like infant formulas, rice, cereals, salt and dairy products. The ferric pyrophosphate data will be essential in fortification of food policy development to fight iron deficiency and anemia in high populations that are susceptible. While excess of chronic iron will cause certain harmful effects that include hepatitis, cancer of liver, cirrhosis, vomiting and diarrhea, intestinal irritation, osteoporosis, articular pain, heart disorder and hormonal disturbance (Schumann, 2001).

2.1 Yoghurt fortification with calcium

This is a point that vitamin D and calcium are essential in decreasing the fracture risk, numerous supplementation studies have scrutinized their effects on bone metabolism indices and bone mass. Due to the fact of Osteoporosis common disease that affect both old men and women that have been related to increase in bone fracture. There is still few clinical trials probing effects of the nutrients when supplemented to vulnerable group population through dairy products fortified. The yoghurt product fortified sensory properties is manipulated by minerals used at high concentration. According to Ocak and Rajendram

(2013) that calcium used should be in micronized type to prevent adverse impact on the sensory properties in this regard. The possible mode to improve mineral levels in products of dairy is attained by micron-zation of minerals that is ease dispersion of ultrafine particles, texture and acceptance of dairy products, improve mouth feel (Ocak and Rajendram, 2013). The yoghurt fortified with calcium is the best decision in the use of tri-calcium citrate that give rise to good nutritional valve and technological properties. In yoghurt and other dairy products tricalcium citrate can be used at a concentration more than 1g/L of calcium (Gerhart and Schottenheimer, 2013), while liquid mineral suspension can also be used with hydrocolloid addition or starch will result in stabilization of this suspensions by decreasing minerals sedimentation. In the worldwide particularly in Europe, where health right on products are controlled by the European food safety authority (EFSA) which is health right control while Zinc and Magnesium proffer several alternatives for fortification of new products theories. The minerals and their several beneficial effects on human health are raising awareness to gain significance in products of dairy as well as calcium and other ingredients that contain nutrition. The problem of scientific will rise with higher levels of mineral fortification, zinc citrate and tri-magnesium will prove their higher use in products of dairy (Gerhart and Schottenheimer, 2013).

2.2 Yoghurt fortification with vitamins

The compounds of vitamins play vital role in the body, products of milk fermented like yoghurt have been thought as sources of vitamin. But due to some starters' culture ability to synthesize B vitamin required for their growth, diverse vitamin contents are present in yoghurt too. The fermented milk products and yoghurt may have diverse vitamins concurring to the culture starter that is used to produce the product (Akın, 2006). Vitamin assessment is also more difficult because methods like incubation time, heat treatment, storage condition and temperature changes the content of vitamin in yoghurt. For proper skeletal development vitamin D is very essential that plays important role in serum phosphorus and calcium concentration in the body regulation. The skin when expose to the solar UV radiation received its Vitamin D due to photosynthesis; therefore Vitamin D is not involved in essential nutrient of the body group. While on the other side when latitudes is above 40° N or 40°S no photosynthesized D vitamin is manufactured by the skin for numerous months of the year; but to prevent deficiency D vitamin supplementation is needed (Ladizesky *et al*., 1995). The vitamin D yielded on the skin as effect of skin exposure to sun is restricted by sunscreen use (Hollis, 2005).The vitamin D deficiency resulted into diseases like osteoporosis, osteomalacia and childhood rickets which also have shown increase of developing cancers risk, autoimmune diseases and osteoporotic fractures (Holick, 2002). Because D vitamins are hormone its' receptor that belongs to thyroid and steroid hormone family and nuclear receptors referee its' genomic action of mechanism. The differentiative, apoptotic and Antiproliferative effects of the D vitamin deficiency are viewed in the cells of prostate cancer in vitro (Ylikomi *et al*., 2002; Schwartz *et al*.,1997; Mantell *et al*.,2000). In epidemiological examinations vitamin D have negative effects on colon cancer (Garland *et al*.,1989) and breast cancer (Grant, 2002), which result also in hypertension, diabetes type I, some other cancers and multiple sclerosis(Holick, 2004). The stability of vitamin D in milk and other dairy products have been conducted by some researches (Renken and Warthesen, 1993; Upreti and Mistry, 2002; Kazmi *et al*., 2007; Wagner *et al*., 2008; Hanson and Metzger, 2010) and all maintained that vitamin D is steady during processing and storage of the products. There is no enough vitamin A and C quantity in low-fat dairy products that are not fortified, normally low fat milk and other dairy products have to be improved with vitamin A and not with vitamin C. Dairy products fortification with vitamins A and C leads to enhancement in their nutritive quality which increases their acceptability properties (Dave and Shah, 1997), high quantities of vitamin A is toxic while pro-vitamins like carotene are not deadly. Report have shown that doses like 25,000 and 40,000-50,000 IUs are deadly for children and adults (Preedy *et al*., 2013). Therefore its' suggested that carotene be used for dairy products fortification, intake of more than 100g/day of vitamin D increases health risk and high doses of more than 250g/day are known to cause kidney damages and tissue damages (Preedy *et al*., 2013) while Hanson and Metzger, (2010) reported that level of 250g/day of vitamin D consumption for 5 months have no adverse effects.

3.0 Bioengineered yoghurt

Bioengineering is the use of engineering principles, practices and technologies to the fields of medicine and biology especially in solving problems and improving care.

The bacteria modified genetically have been use for long as vectors of gene delivery mainly by intravenous administration some developing by clinical trials. Some molecules bioengineered into *Lactobacilli* strains include the following interleukin-10, cyanovirin, cytosine deaminase, extracellular domain of TRAIL and betacarotene. Food grade discovery that bacteria vector can be consumed and then can be transfer out of gastrointestinal tract to deliver the gene of interest successfully is new (Cronin *et al*., 2010). The investigation proposes that bacteria in yoghurt

that are genetically engineered should be consumed only in adequate quantities to deliver the products of the engineered genes systematically. Also the facultative anaerobic bacteria and anaerobic bacteria mainly in yoghurt specifically colonize necrotic and hypoxic regions like those that are found in tumors though other methods are also active in tumor homing (Baban *et al*., 2010).

The below table 1 gives the quantity of the mineral and Vitamins levels suggested in literature for several groups that are vulnerable among population of beneficiaries of food aid involving children suffering from moderate acute malnutrition (MAM), non-breast fed (NBF) and breast-fed(BF) infants, Older children and adults involving lactating and pregnant women(older & LP) and a composite group comprises of infants, young children and lactating and pregnant women.

a. Breast fed-infants age of 6-23months
b. Children suffering from moderate acute malnutrition: The recommendation is for age with highest requirement for nutrition density, the Data is presented on per 1000kcal basis have been converted to per 100g FBF basis using kcal density of USAID CSB.
c. Non-breast –fed infants: the recommendation is for age with requirement for highest nutrition density.
d. Older children and adults involving lactating and pregnant women: the recommendation is for group with requirement for highest nutrient density.
e. The composite vulnerable group includes infants, young children, lactating and pregnant women

Table 1: Production of Fortified and Bioengineered Yoghurt around the globe (per 100g food)

Micronutrient	Units	BF infant[a]	MAM child[b]	NBF infant[c]	Older and LP[d]	Composite[e]
Biotin	mg	2.9	4.9	_	_	_
Choline	mg	91.8	83.8	_	_	_
Folic acid	mg	83	131.6	156	273	95
Niacin	mg	6.1	6.8	7.3	8.2	9.74
Pantothenic acid	mg	0.7	1.1	3.3	2.7	3.53
Riboflavin	mg	0.36	0.68	0.73	0.64	0.967
Thiamin	mg	0.36	0.38	0.55	0.64	0.746
Vitamin A	mg	500	714.4	734	377	154
Vitamin B6	mg	0.44	0.68	0.55	0.87	0.752
Vitamin B12	mg	0.52	0.98	1.27	1.29	1.5
Vitamin C	mg	140-280	37.6	55	28	40
Vitamin D3	mg	2-4	4.1	9.2	8.1	25
Vitamin E	mg	10	8.3	5.2	4.7	10.88
Vitamin K	mg	_	15.0	_	_	33
Calcium	mg	200-400	315.8	734	698	353
Copper	mg	400-800	3334.6	_	_	390
Iodine	mg	180	75.2	164	113	230
Iron	mg	14	6.8	17.1	11.6	15.5
Magnesium	mg	80-120	112.8	99	111	94
Manganese	mg	1200	451.2	_	_	790
Phosphorus	mg	150-200	338.4	504	606	513
Potassium	mg	_	601.6	2654	2699	707
Selenium	mg	20	20.7	18.3	18.4	20
Sodium	mg	_	206.8	219	327	239
Zinc	Mg	8.3	7.5	15.4	8	6.85

Keys:
MAM- Moderate acute malnutrition; NBF- non-breast fed; BF- breast fed; older & LP- Older children and adults involving lactating and pregnant women.
Sources: Fleige *et al*., 2010; Webb *et al*., 2011; Lutter and Dewey, 2003; Golden, 2009

4.0 Bacteriotherapeutic effects of yoghurt probiotic

The microorganisms called probiotics are vital microorganisms, when ingested in sufficient quantities give a benefits to the host health (FAO/WHO 2002), the positive health effect observed on its' consumption required minimum level of the probiotic microorganisms and the level depend on the used strains and the health effect required normally is between 10^{11}cfu/d (Vanderhoof, 2008). The dairy products fermented like yoghurt have the capacity to

aid viability of probiotic organism cultures that provide proper medium for release into the body system which are combined easily into diets. The yoghurt products have been prepared progressively with probiotic organisms like *Lactobacillus acidophilus* with changing viability over range of the shelf life (Lourens-Hattingh and Viljoen, 2001). The intestinal bacteria are non-pathogenic that play essential role in functioning of normal gut that enhance disease resistance, but factors like diet, lifestyle changes, stress increase and antibiotic medications consumption can lead all to the gut bacteria balance changes (Savino *et al*., 2007). As the number of beneficial bacteria e.g *Bifidobacteria* and *Lactobacilli* reduces while number of pathogenic bacteria increase can cause complaints of health and infections that can pre-dispose persons to numbers of life threatening diseases like inflammatory bowel diseases and bowel cancer (Reeta *et al*., 2015). In this type of conditions, probiotic bacteria will exercise a positive effect on the consumers' health by rectifying health promoting balance and gastrointestinal tract bacteria (Ouwehand *et al*., 1999).The Lactic acid bacteria are the microorganisms normally used as probiotics like *Bifidobacteria* and *Lactobacilli* that are both naturally found in gut. Many health benefits connected to use of probiotic bacteria include the following

4.1 Effects of bioengineered and fortified yoghurt probiotic on immunologic effects

Immune system is liable for protecting human from disease and infection, while sub groups of people can have weak immune system function like surgical patients, elderly, infants, HIV positive individuals, trauma victims and people under stress that reduces natural protection from their disease (Van Loo *et al*., 2005). The probiotic microorganisms have connection to enhancing immune system function in this groups (Hatakka *et al*., 2001) and human investigation results show that bacteria probiotic strains present in yoghurt improve immunity that also reduce incidences conditions like gastrointestinal disorders and symptoms of allergy, cancer though more investigation is needed.

4.1.1 Diarrhea

The diarrhea occurs regularly from disturbance to natural balance from micro-flora of the gut during an infection with pathogenic bacteria. The diarrhea treatment with probiotics microorganisms have been traditionally one of the major areas for probiotics use. The evidence documented is the exists for the duration of diarrhea form in children known as Rotavirus diarrhea is shortening by certain probiotic (Marteau and Boutron-Ruault, 2002). The diverse strains of bacteria such as *Lactobacillus rhamnosus GG, Lactobacillus reuteri* SD2112, *Lactobacillus casei* DN-114 001 and *Saccharomyce cerevisiae* strain of *bolardii* tested in clinical trials controlled reduces duration and severity in diarrhea. The controlled clinical trials on some meta-analysis have been published which conclude that these probiotics are effective and safe (Ouwehand *et al*., 1999). The treatment and prevention of diarrhea after antibiotics used especially in elderly have been linked with particular strains of probiotic like *Lactobacillus bulgaricus, Lactobacillus acidophilus and B.longum* (Szajewska *et al*., 2001) as shown in Table 2.

Table 2: Application of fortified and Bioengineered Yoghurt as Bacterio-therapy function

NCT number	Malignancy	N	Objective	intervention	Outcome measures
NCT02944617	Renal cell cancer	20	To prevent diarrhea in patients treated with sunitinib by probiotics	Micronutrient fortified probiotic yoghurt	Changes in level of *Bifidobacterium* spp. Assessed in stool samples
NCT01790035	GIneoplasms Riehl *et al*., 2018	23	To prevent chemoradiation induced toxicity by probiotics	*L.rhamnosus* GG	Efficacy, safety, diarrhea subscale score

NCT number	Malignancy	N	Objective	intervention	Outcome measures	
NCT02351089	Gynecologic cancer	200	To assess the efficacy of probiotics in preventing GI toxicity associated with radiation of gynecologic cancer.	Capsules probiotic powder and corn starch	Change inincidence of loose or watery stools	
NCT03552458	Head and neck cancer	50	To assess the role of probiotics in preventing oral mucositis	*Lactobacillus reuteri* oral solution (BioGaia)	Oral mucositis severity, oral bacteria genetic and transcription analysis.	
NCT02771470	Lung cancer Tian *et al.*, 2019	41	To assess the effects of chemotherapy on microbiome and probiotics on chemotoxicity	*Clostridium butyricum*	Composition of microbiome with probiotics, adverse effects of chemo, change in immunity and nutrition index	China
NCT01723592	Breast cancer	27	To improve the quality of vaginal flora by probiotics	*L.rhamnosus, L.jensenii, L.crispatus, L.gasseri*	Isolation of specific Lactobacilli from vagina	Austra

Sources: Naito *et al*., 2008; Mego *et al*., 2013; Panebianco *et al*., 2018

Table 3: Application of fortified and bioengineered yoghurt for Nutrient deficiency management

Components	Unit	Full fat milk	Full fat yoghurt	Non-fat milk	Non-fat yoghurt
Energy	Kcal	68	70	35	39
Protein	G	3.3	3.8	3.5	4.4
Fat	G	3.8	3.8	0.1	0.1
Lactic acid	G	0	0.8	0	1.0
Potassium	mg	157	157	150	187
Calcium	mg	120	120	123	143
Phosphorus	mg	92	92	97	109
Magnesium	mg	12	12	14	14
Sodium	mg	48	48	53	57
Iodine	mg	0.46	0.46	0.45	0.44
Chlorine	mg	102	102	100	121
Retinol	µg	52	28	1	8

Components	Unit	Full fat milk	Full fat yoghurt	Non-fat milk	Non-fat yoghurt
Carotene	µg	21	21	Tr*	5
Vitamin B1	µg	30	60	40	50
Vitamin B2	µg	170	270	170	250
Vitamin B6	µg	60	100	60	90
VitaminB12	µg	0.4	0.2	0.4	0.2
Vitamin C	mg	1	1	1	1
Vitamin D	µg	0.03	0.04	Tr*	0.01
Vitamin E	µg	90	50	Tr	10
Folic acid	µg	6	18	5	17
Nicotinic acid	µg	100	200	100	100
Pantothenic acid	µg	350	500	320	450

Tr: trace

Table 4: Diverse varieties of yoghurt Nutrition composition per 100g

Composition	Whole milk yoghurt	Low fat yoghurt	Non-fat yoghurt	Greek-style yoghurt	Drinking yoghurt
Energy(kcal)	79	56	54	133	62
Protein(g)	5.7	4.8	5.4	5.7	3.1
Carbohydrate(g)	7.8	7.4	8.2	4,8	13.1
Fat(g)	3.0	1.0	0.2	10.2	Trace
Thiamin(mg)	0.06	0.12	0.04	0.12	0.03
Riboflavin(mg)	0.27	0.22	0.29	0.13	0.16
Niacin(mg)	0.2	0.1	0.1	0.1	0.1
VitaminB6(mg)	0.10	0.01	0.07	0.01	0.05
VitaminB12(mg)	0.2	0.3	0.2	0.2	0.2
Folate(µg)	18	18	8	6	12
Carotene(µg)	21	Trace	Trace	Trace	Trace
Vitamin D	0.01	Trace	0.1	Trace	Trace
Potassium(mg)	280	228	247	184	130
Calcium(mg)	200	162	160	126	100
Phosphorus(mg)	170	143	151	138	81

Source: Dairy council 2013; Akın 2006, Hashemi *et al*., 2015

4.2 Nutrient deficiency management

The milk fermented products called yoghurt is very nutritious and easy to digest that contain vital source of nutrients and some accurate vitamin and minerals. Yoghurt nutrition composition can differ base on the starter culture strains used in fermentation process, milk type used, type of species milk is obtained from, milk solid type, solid milk non-fat, sweeteners and fruits type additional to milk earlier, then fermentation and also fermentation process span. Yoghurt consumption help to manage nutrition deficiency in human as a result of its nutrition composition as shown in Table 4 below on diverse varieties of yoghurt. From table 3 below the recommended yoghurt to managed malnutrition is non-fat yoghurt due to its nutrition composition (Dennis *et al*., 2013).

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

Not Applicable.

Competing interests

All authors declare no competing interests.

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Conclusion

The most healthy and nutritious fermented milk products consumed around the world is yoghurt, which is a suitable prospective that offer to carry nutritious ingredients to human diet. This review studies reveals that peoples suffering from micronutrient deficiency, can take enriched food products like yoghurt to reduce the mal-nutritional diseases vividly when they consumed fortified and bioengineered yoghurt. This study shows different nutrition composition of fortified and bioengineered products and their effect of enriched on food products to inhibit disease. This review paper reveals that enhancement of food can treat or prevent most diseases, in young children and immune-compromised population, which would have

general important effect on enhancing the community health.

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